

**Supplementary Information:**

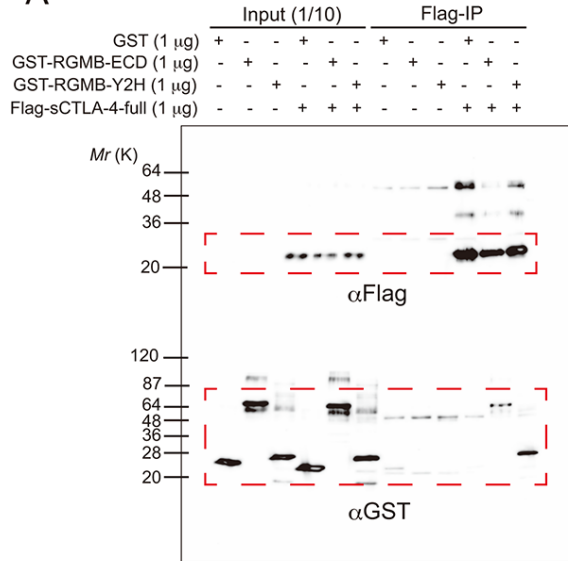
**RGMB enhances the suppressive activity of the monomeric secreted form of CTLA-4**

Takashi Sekiya\* and Satoshi Takaki

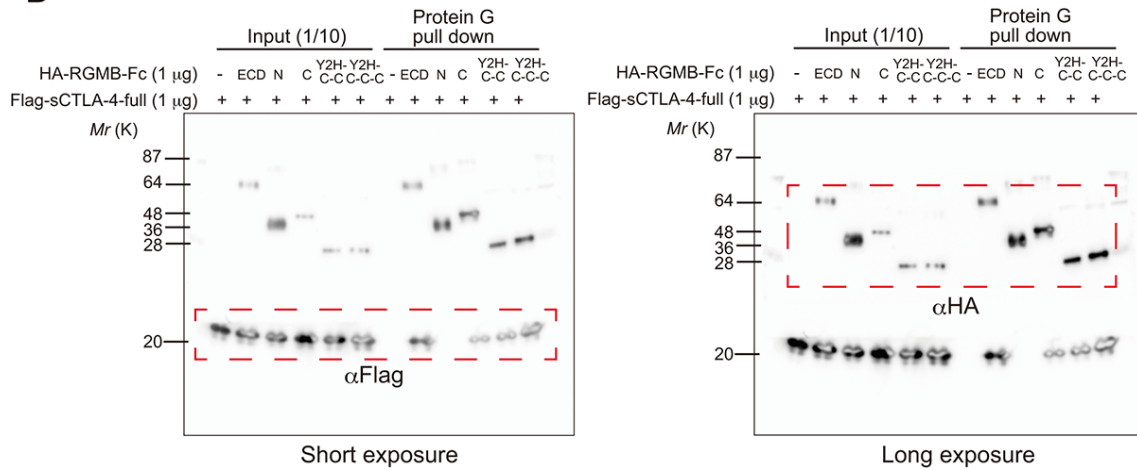
\*Correspondence: Corresponding Author: lb-sekiya@hospk.ncgm.go.jp

Unprocessed immunoblot images

**A**

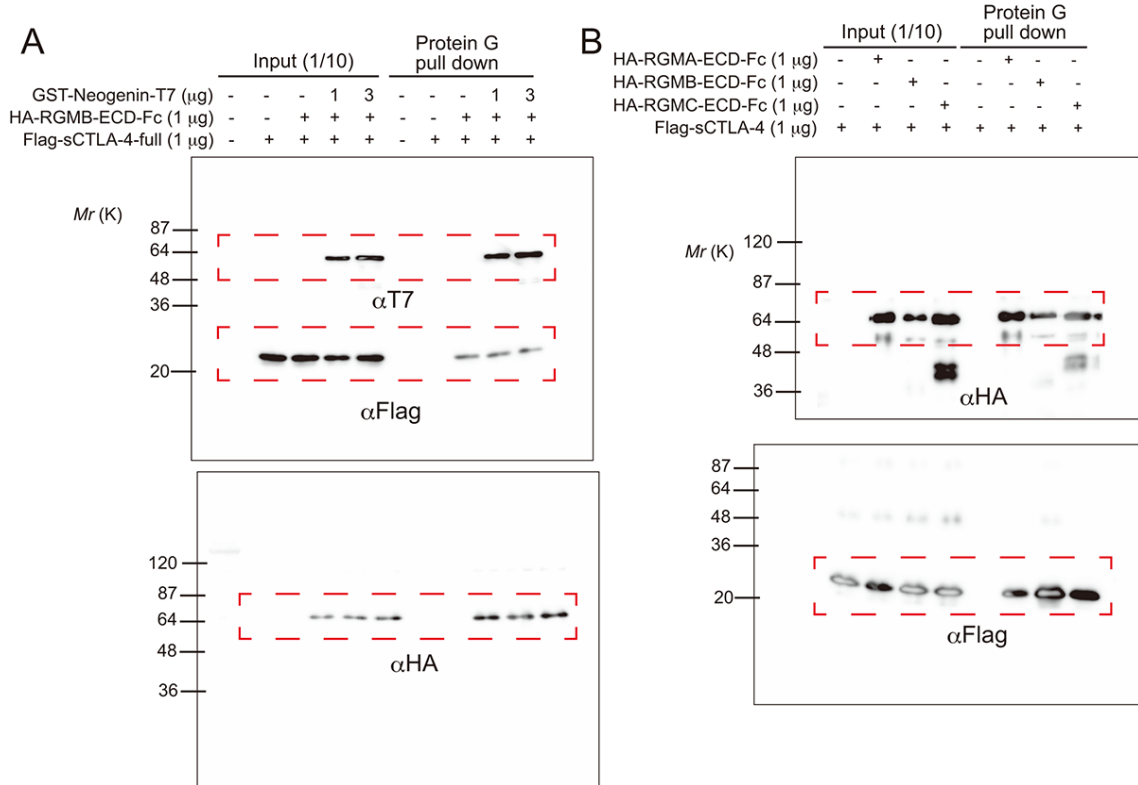


**B**

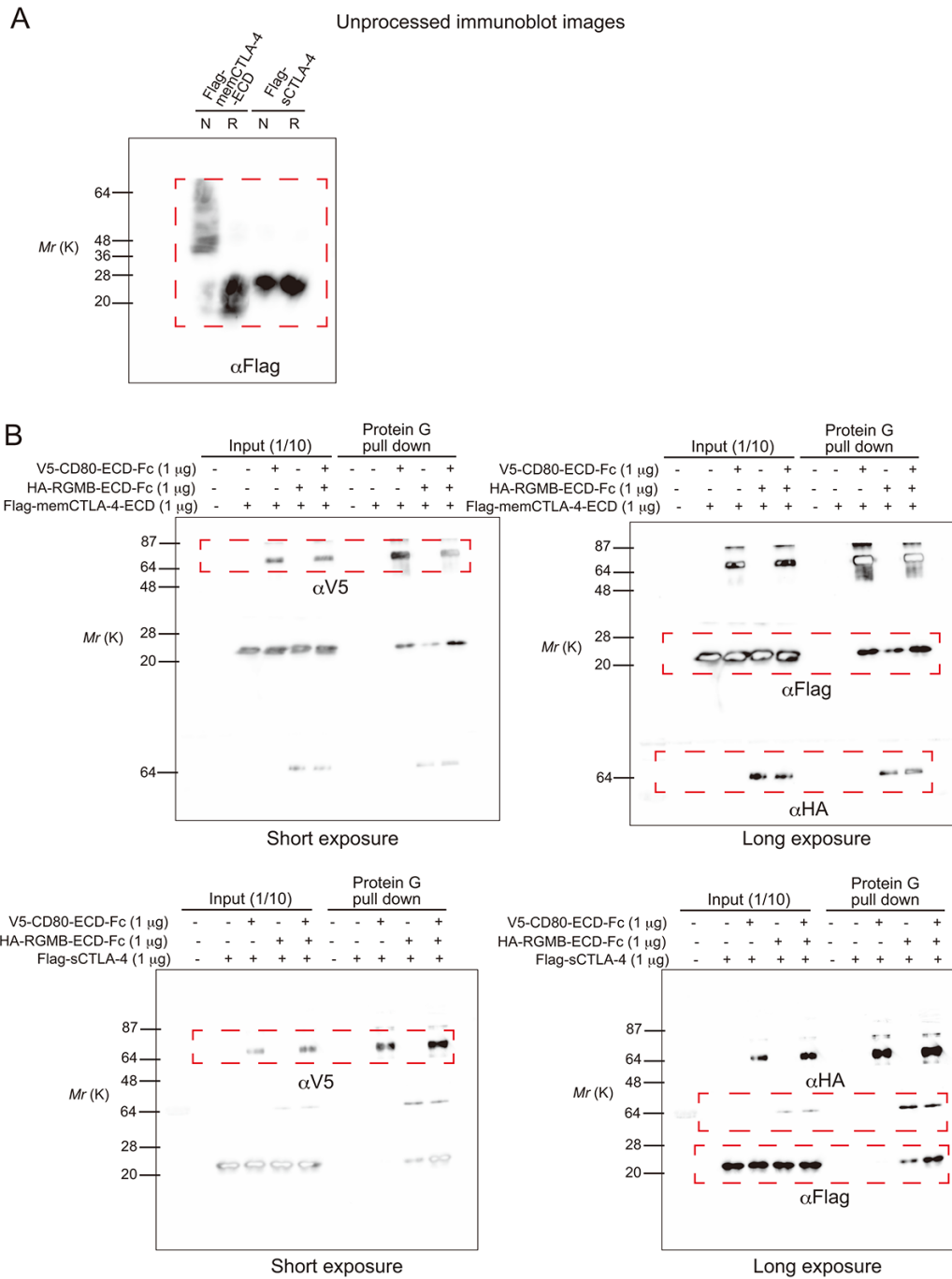


**Supplementary Figure S1: Uncropped versions of all Western blots shown in Fig. 1.** (A),(B) shows uncropped versions of Western blots shown in Fig. 1A and 1C, respectively. Cropped areas for the main figures are indicated with the dashed lines.

Unprocessed immunoblot images

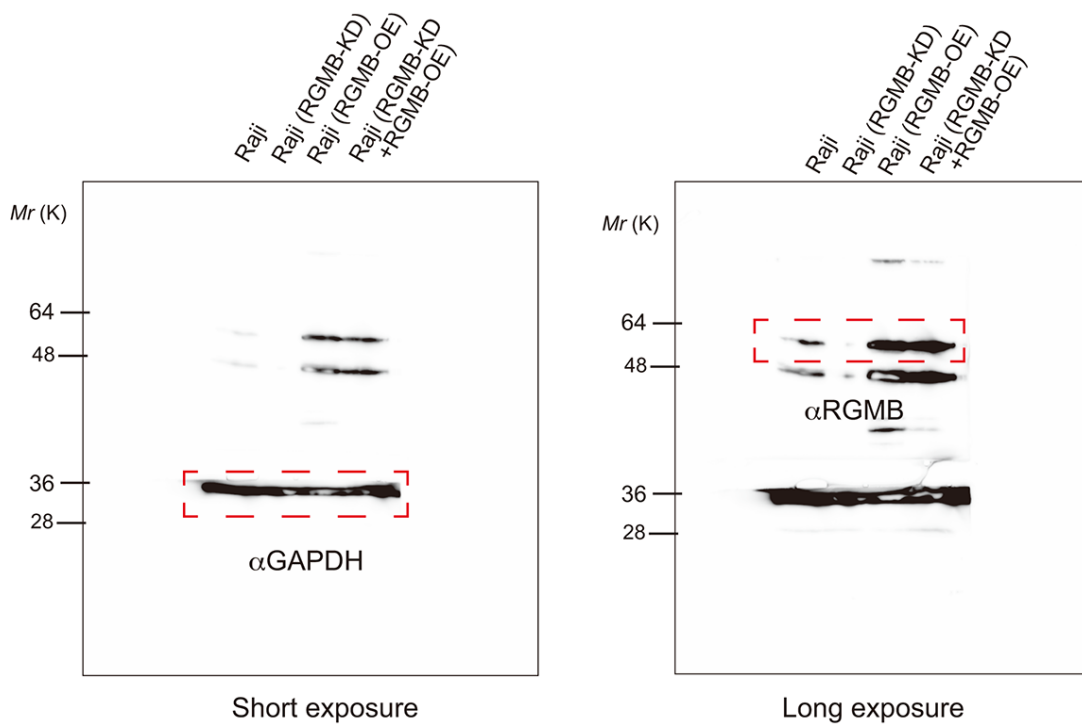


**Supplementary Figure S2: Uncropped versions of all Western blots shown in Fig. 2.**  
**(A),(B)** shows uncropped versions of Western blots shown in **Fig. 2A** and **2C**, respectively.  
 Cropped areas for the main figures are indicated with the dashed lines.



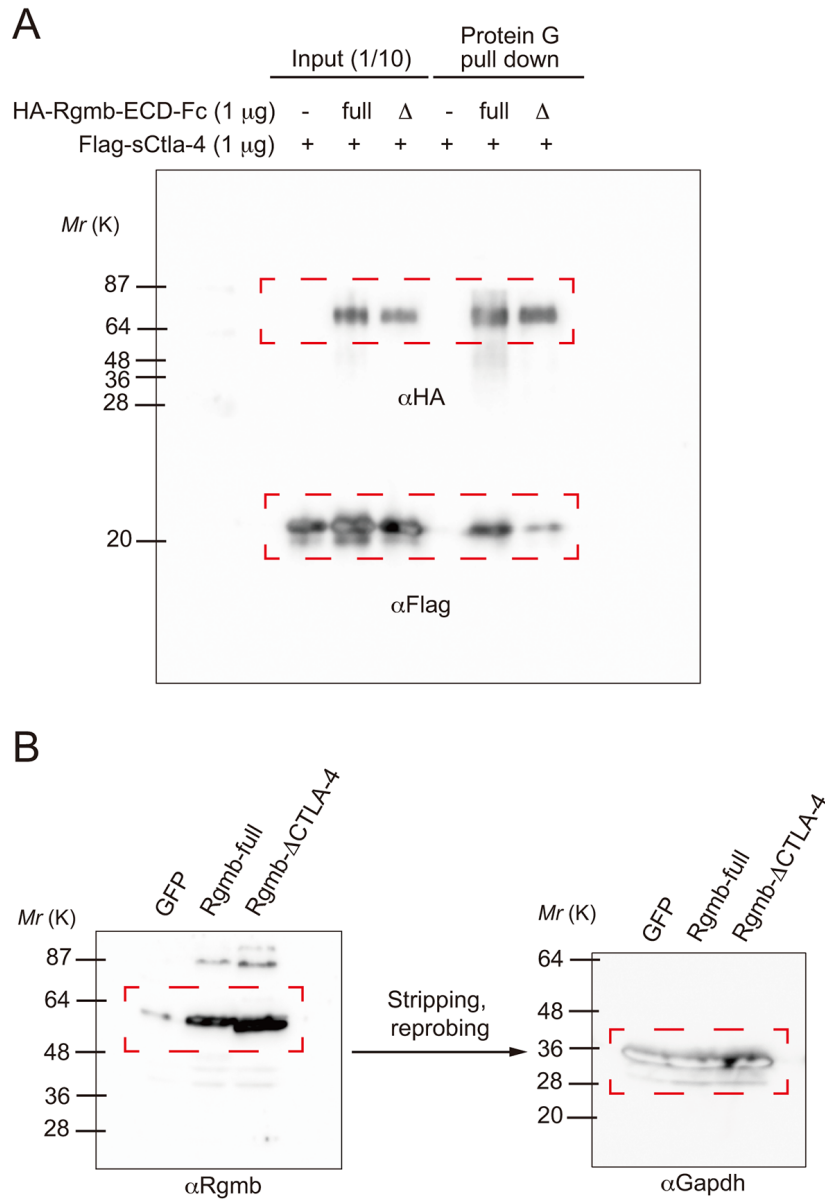
**Supplementary Figure S3: Uncropped versions of all Western blots shown in Fig. 4.** (A),(B) shows uncropped versions of Western blots shown in Fig. 4A and 4B, respectively. Cropped areas for the main figures are indicated with the dashed lines.

Unprocessed immunoblot images



**Supplementary Figure S4: Uncropped versions of all Western blots shown in Fig. 5.**

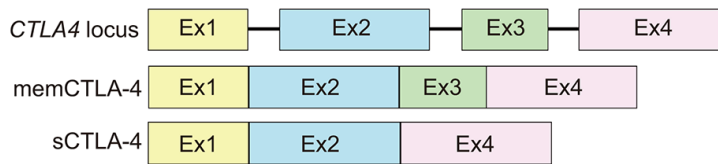
Unprocessed immunoblot images



**Supplementary Figure S5: Uncropped versions of all Western blots shown in Supplementary Fig. S7.**

(A),(B) shows uncropped versions of Western blots shown in **Supplementary Fig. S7B** and **S7E**, respectively. Cropped areas for the main figures are indicated with the dashed lines.

A



B

Estimation of proportion of sCTLA-4 in total CTLA-4 products

Method 1. from exon junction reads:



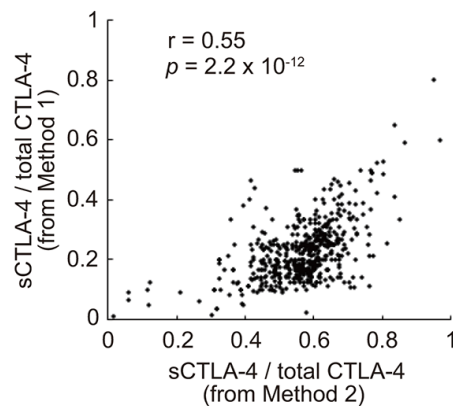
$$\text{sCTLA-4} / \text{total CTLA-4} = \text{Junction Ex2:Ex4} / (\text{Junction Ex2:Ex3} + \text{Junction Ex2:Ex4})$$

Method 2. from total exon reads:



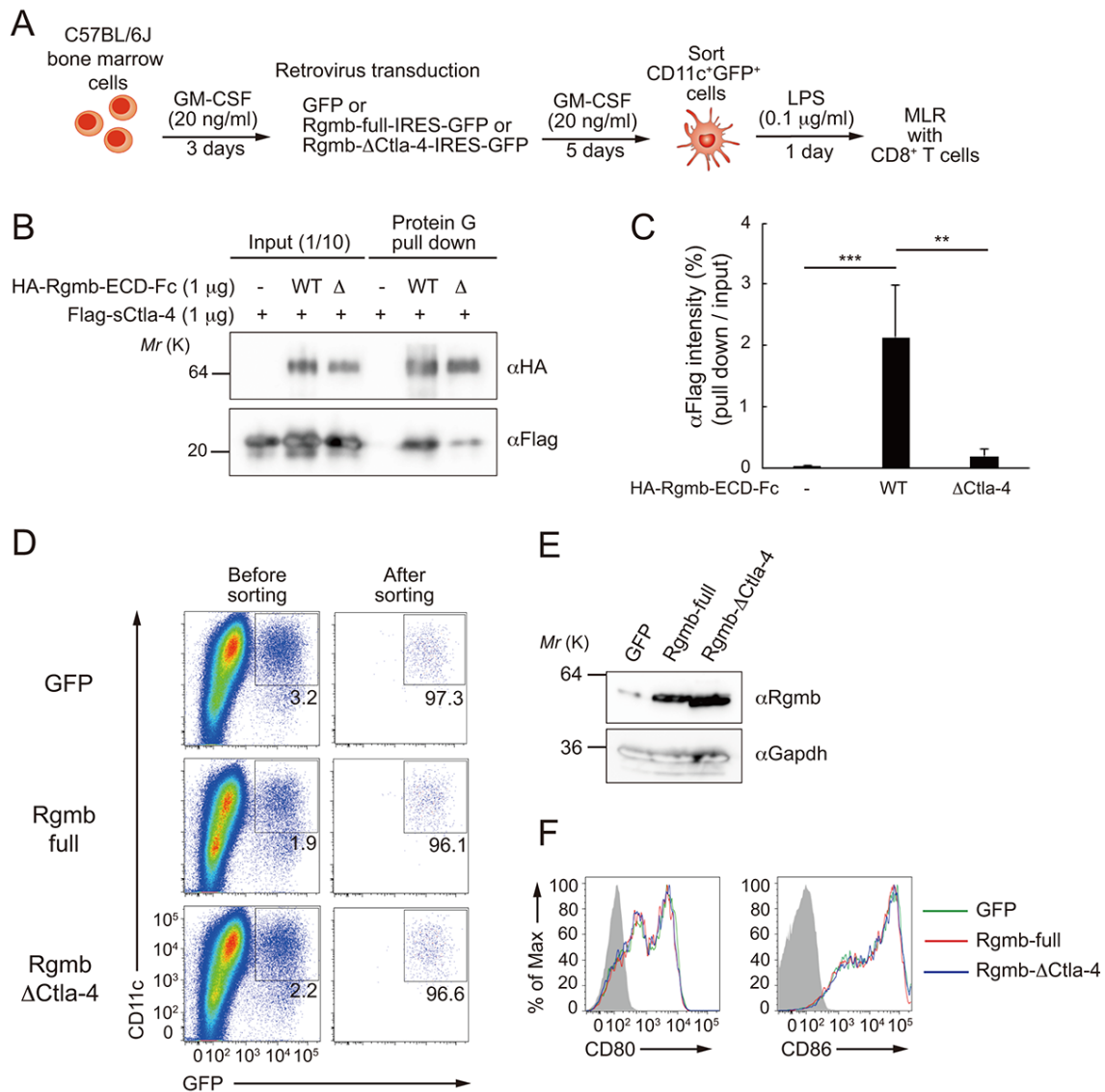
$$\text{sCTLA-4} / \text{total CTLA-4} = (\text{Total Ex2} - \text{Total Ex3}) / \text{Total Ex2}$$

C



**Supplementary Figure S6: A method to determine the expression levels of sCTLA-4 in TCGA datasets.**

(A) Exon-intron structure of *CTLA4*. (B) Schematics of the methods that determine the proportion of sCTLA-4 in total CTLA-4 products. (C) Correlation between the proportions of sCTLA-4 in total CTLA-4 products determined by the Method 1 and the Method 2 shown in (B). Samples which have more than 1 read at both exon2:exon3 and exon2:exon4 junctions were pooled from 33 non-hematologic tumor samples and used in this analysis (n=521). Spearman's correlation coefficient and *p* value are shown.



### Supplementary Figure S7: Construction of Rgmb-overexpressing BMDCs.

(A) Schematic of experiments shown in Fig. 6. (B) Association of mouse Rgmb with mouse sCtla-4 *in vitro*. Mammalian cell-produced full-length sCtla-4 (1 μg) was incubated with 1 μg of mammalian cell-produced extracellular domain of wildtype Rgmb (WT) or of deleted form of Rgmb which lacks a region corresponding to the CTLA-4 interaction domain of human RGMB (Δ). HA-Rgmb-ECD-Fc was pulled-down with protein G Sepharose, and the bound proteins were subjected to immunoblotting analysis with the antibodies indicated. Full-length blots are shown in Supplementary Fig. S5A. (C)

Quantification of the results in **(B)**. Intensities of the bands of pulled-down Flag-sCtla-4, relative to the intensities of the input bands, are shown. **(D)** Flow cytometry of CD11c and GFP expression in BMDCs, which were transduced with retrovirus which express GFP, full length Rgmb, or deleted Rgmb which lacks the Ctla-4 interaction region, whose expression can be monitored by IRES-mediated co-expression of GFP. Indicated areas in the left panels were sorted and supplied for MLR experiment shown in **Fig. 6**. **(E)** Western blot analysis of the expression levels of Rgmb and Gapdh in the indicated cells. Full-length blots are shown in **Supplementary Fig. S5B**. **(F)** Flow cytometry of the indicated molecules in the indicated cells. Shaded area represent the isotype-matched control. Scores show means  $\pm$  SD of data obtained from three independent biological replicates. \* $p < 0.05$  (one-way ANOVA with Bonferroni test).